L1 ANSWER 3 OF 96 COPYRIGHT 1993 NLM
AN 92035562 MEDLINE
TI Multianalyte microspot immunoassay--microanalytical "compact disk" of the future.
AU Ekins RP; Chu FW
CS Department of Molecular Endocrinology, University College and Middlesex School of Medicine, London, U.K.)
SO Clin Chem, (1991 Nov) 37 (11) 1955-67 Ref: 29
Journal code: DBZ ISSN: 0009-9147
CY United States (Z1.107.567.875.)
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals; Cancer Journals
EM 9202
AB Throughout the 1970s, controversy centered both on immunoassay "sensitivity" per se and on the relative sensitivities of labeled antibody (Ab) and labeled analyte methods. Our theoretical studies revealed that RIA sensitivities could be surpassed only by the use of very high-specificity nonisotopic labels in "noncompetitive" designs, preferably with monoclonal antibodies. The time-resolved fluorescence methodology known as DELFIA--developed in collaboration with LKB/Wallac--represented the first commercial "ultrasensitive" nonisotopic technique based on these theoretical insights, the same concepts being subsequently adopted in comparable methodologies relying on the use of chemiluminescent and enzyme labels. However, high-specific-activity labels also permit the development of "multianalyte" immunoassay systems combining ultrasensitivity with the simultaneous measurement of tens, hundreds, or thousands of analytes in a small biological sample. This possibility relies on simple, albeit hitherto-unexploited, physicochemical concepts. The first is that all immunoassays rely on the measurement of Ab occupancy by analyte. The second is that, provided the Ab concentration used is "vanishingly small," fractional Ab occupancy is independent of both Ab concentration and sample volume. This leads to the notion of "ratiometric" immunoassay, involving measurement of the ratio of signals (e.g., fluorescent signals) emitted by two labeled Abs, the first (a "sensor" Ab) deposited as a microspot on a solid support, the second (a "developing" Ab) directed against either occupied or unoccupied binding sites of the sensor Ab. Our preliminary studies of this approach have relied on a dual-channel scanning-laser confocal microscope, permitting microspots of area 100 microns 2 or less to be analyzed, and implying that an array of 10(6) Ab-containing microspots, each directed against a different analyte, could, in principle, be accommodated on an area of 1 cm2. Although

measurement of such analyte numbers is unlikely ever to be required, the ability to analyze biological fluids for a wide spectrum of analystes is likely to transform immunodiagnostics in the next decade.

L1 ANSWER 5 OF 96 COPYRIGHT 1993 NLM
AN 91182922 MEDLINE
TI Multi-analyte immunoassay.
AU Ekins RP
CS Department of Molecular Endocrinology, University College and Middlesex School of Medicine, London, UK.)
SO J Pharm Biomed Anal, (1989) 7 (2) 155-68 Ref: 21
Journal code: A2C ISSN: 0731-7085
CY England: United Kingdom (Z1.542.363.300.)
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 9107
AB Immunoassays rely on the molecular recognition properties possessed by antibodies to measure substances defined by a particular structure. They can therefore be defined as "structurally specific", as distinct from "functionally specific" assays, e.g. bioassays, which compare the biological effects of substances which are functionally similar, but which may differ in molecular structure. Within the broad class of "immunoassays", two subclasses may be distinguished, differing in their design. These may be described as "competitive" and "non-competitive", respectively, reflecting their dependence on the use of optimal concentrations of antibody which are either very small or very large. It is demonstrable that "non-competitive" assays are those relying on measurement of occupied antibody binding sites following reaction with analyte; conversely "competitive" assays rely on measurement of unoccupied sites. In certain assay designs, it may be shown that fractional antibody binding site occupancy is independent of (a) antibody concentration, and (b) sample volume. Such assays may be termed "ambient analyte immunoassays". This concept has been exploited in the development of free hormone and drug assays, and currently underlines the development of salivary "dip-stick" assays in the Author's Department. The concept is also being exploited in the development of "multi-analyte" immunoassay systems, enabling the simultaneous measurement of tens or even hundreds of substances simultaneously in the same small sample. These systems depend on measurement of fractional antibody occupancy using two different labels: one labeling the "sensing" antibody, the second labeling a "developing

antibody", selected to react either with occupied or unoccupied sites on the "sensing" antibody. The ratio of signals emitted by the two labeled antibodies reveals the analyte concentration to which the sensing antibody has been exposed. An array of sensing antibodies, each labeled with the same fluorescent label, is scanned (by a laser), and the fluorescent signal ratio emitted from each discrete antibody couplet in the array measured. Multi-analyte immunoassay systems of this kind are likely to totally transform medical diagnosis in the foreseeable future and are also likely to be of value in the analysis of complex protein mixtures deriving from recombinant DNA technologies.

L1 ANSWER 17 OF 96 COPYRIGHT 1993 NLM
AN 87167266 MEDLINE
TI Principles of free hormone measurement.
AU Ekins RP; Jackson T; Sinha A; Edwards P
SO J Endocrinol Invest, (1986) 9 Suppl 4 3-15 Ref: 40
Journal code: IAM ISSN: 0391-4097
CY Italy (Z1.542.489.)
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LA English
FS Priority Journals
EM 8707

L2 ANSWER 6 OF 144 COPYRIGHT 1993 ACS
AN CA114(9):77971x
TI Fluorescence spectroscopy and its application to a new generation of high sensitivity multi-microspot, multianalyte, immunoassay
AU Ekins, Roger; Chu, Frederick; Biggart, Elizabeth
CS Dep. Mol. Endocrinol., Univ. Coll.
LO London W1N 8AA, UK
SO Clin. Chim. Acta, 194(1), 91-114
SC 9-0 (Biochemical Methods)
DT J
CO CCATAR
IS 0009-8981
PY 1990
LA Eng
AB A review with 14 refs. Advantages of high sensitivity multi-microspot multianalyte immunoassay systems are discussed.

L2 ANSWER 10 OF 144 COPYRIGHT 1993 ACS
AN CA113(7):52561e
TI Measurement of free hormones in blood
AU Ekins, Roger
CS Middlesex Sch. Med., Univ. Coll.

LO London W1N 8AA, UK
SO Endocr. Rev., 11(1), 5-46
SC 2-0 (Mammalian Hormones)
DT J
CO ERVIDP
IS 0163-769X
PY 1990
LA Eng
AB A review, with 226 refs., on the detn. of free hormones in blood and the biol. significance of such unbound hormones.

L2 ANSWER 15 OF 144 COPYRIGHT 1993 ACS
AN CA112(1):3922u
TI High specific activity chemiluminescent and fluorescent markers: their potential application to high sensitivity and 'multi-analyte' immunoassays
AU Ekins, Roger; Chu, Frederick; Micallef, Jacob
CS Univ. Coll., Univ. London
LO London W1N 8AA, UK
SO J. Biolumin. Chemilumin., 4(1), 59-78
SC 9-10 (Biochemical Methods)
DT J
CO JBCHE7
IS 0884-3996
PY 1989
LA Eng
AB The sensitivities of immunoassays relying on conventional radioisotopic labels, i.e., RIA (RIA) and immunoradiometric assay (IRMA), permit the measurement of analyte concns. .gtorsim.107 mols./mL. This limitation primarily derives, in the case of competitive or limited reagent assays, from the manipulation errors arising in the system combined with the physicochem. characteristics of the particular antibody used; however, in the case of noncompetitive systems, the specific activity of the label may play a more important constraining role. It is theor. demonstrable that the development of assay techniques yielding detection limits significantly <107 mols./mL depends on the following: (1) the adoption of noncompetitive assays designs; (2) the use of labels of higher specific activity than radioisotopes; and (3) highly efficient discrimination between the products of the immunol. reactions involved. Chemiluminescent and fluorescent substances are capable of yielding higher specific activities than commonly used radioisotopes when used as direct reagent labels in this context, and both thus provide a basis for the development of ultrasensitive, noncompetitive, immunoassay methodologies. Enzymes catalyzing chemiluminescent reactions or yielding fluorescent reaction products

can likewise be used as labels yielding high effective specific activities and hence enhanced assay sensitivities. A particular advantage of fluorescent labels (albeit one not necessarily confined to them) lies in the possibility they offer of revealing immunol. reactions localized in microspots distributed on an inert solid support. This opens the way to the development of an entirely new generation of ambient analyte microspot immunoassays permitting the simultaneous measurement of tens or even hundreds of different analytes in the same small sample, using (for example) laser scanning techniques. Early experience suggests that microspot assays with sensitivities surpassing that of isotopically based methodologies can readily be developed.

L2 ANSWER 16 OF 144 COPYRIGHT 1993 ACS
AN CA111(23):211547g
TI A method, device, and kit for determination of ambient concentration of several analytes
AU Ekins, Roger Philip
LO UK
SO PCT Int. Appl., 30 pp.
PI WO 8901157 A1 9 Feb 1989
DS W: AT, AU, BR, CH, DE, DK, FI, GB, HU, JP, KR, NL, NO, SE, SU, US
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE
AI WO 88-GB649 5 Aug 1988
PRAI GB 87-558 6 Aug 1987
GB 88-3000 10 Feb 1988
IC ICM G01N033-543
ICA G01N033-78; G01N033-76
SC 9-10 (Biochemical Methods)
SX 2
DT P
CO PIXXD2
PY 1989
LA Eng
AB A method for detg. the ambient concns. of a plurality of analytes in a liq. sample of vol. V L, comprises loading different binding agents, each being capable of reversibly binding an analyte which is or may be present in the liq. sample and is specific for that analyte as compared to the other components of the liq. sample, onto a support at spaced-apart locations such that each location has .ltoreq.0.1, preferably .ltoreq.0.01 V/K, moles of a single binding agent, where K L/mol is the equil. const. of the binding agent for the analyte; contacting the loaded support with the liq. sample to be analyzed, such that each of the spaced-apart locations is contacted in the same operation with the liq. sample, the amt. of liq. used in the sample being such that only an insignificant

SC 9-0 (Biochemical Methods)
DT J
CO CBREEU
IS 0159-8090
PY 1987
LA Eng
AB A review with 43 refs. on improvements in sensitivity of immunoassay techniques.

L2 ANSWER 42 OF 144 COPYRIGHT 1993 ACS
AN CA102(19):161168a
TI Free ligand assay
AU Ekins, Roger Philip; Jackson, Thomas Michael
LO UK
SO PCT Int. Appl., 20 pp.
PI WO 8500226 A1 17 Jan 1985
DS W: JP, US
RW: AT, BE, CH, DE, FR, GB, LU, NL, SE
AI WO 84-GB220 22 Jun 1984
PRAI GB 83-17124 23 Jun 1983
IC ICM G01N033-53
ICS G01N033-78
SC 2-1 (Mammalian Hormones)
DT P
CO PIXXD2
PY 1985
LA Eng
AB A method for measuring the concn. of a free ligand (such as thyroid hormones and other hormones) in a biol. fluid contg. the free ligand and ligand bound to an endogenous binding agent is devised by (1) mixing a fluid sample with an analog of the ligand, a specific binder with which the free ligand and analog bind, and an exogenous binding agent which binds only the analog, with either the ligand or the specific binder being labeled; (2) incubating the resulting mixt. so that the ligand and analog compete for the specific binder; (3) detg. either the amt. of the labeled analog bound to the specific binder or the exogenous binding agent or the amt. of labeled specific binder bound, or not bound, to the ligand analog; and (4) correlating the detd. amt. to the amt. of free ligand present in the sample. Thus, an analog of T4 [51-48-9] suitable for the immunoassay of free T4 was prepd., and an antibody against this analog was produced. The analog was then radiolabeled with 125I. A specific antibody against T4 with an equal affinity for the T4 analog was coupled to solid particles. A mixt. was prepd. of 0.5 mL of a suspension of the solid-phase antibody reagent, 0.5 mL of the [125I]T4 analog (2 nM), and 100 .mu.L of normal human serum. The

proportion of any analyte present in the liq. sample becomes bound to the binding agent specific for it, and measuring a parameter representative of the fractional occupancy by the analytes of the binding agents at the spaced apart locations by a competitive or noncompetitive assay technique using a site-recognition reagent for each binding agent capable of recognizing either the unfilled binding sites or the filled binding sites on the binding agent, said site-recognition reagent being labeled with a marker enabling the amt. of said reagent in the particular location to be measured. A device and kit for use in the method are also provided. A microtiter plate was prepd. contg. spots of Texas Red-labeled antibodies to thyroxine, TSH, and triiodothyronine in each of the wells. The plate was used to measure thyroxine, TSH, and triiodothyronine levels in serum from human patients. The developing antibody for the TSH assay was a 2nd antibody labeled with FITC. The site recognition reagents for the other 2 assays were thyroxine and triiodothyronine coupled to poly-lysine and labeled with FITC. The results correlated well with those obtained by other methods.

L2 ANSWER 18 OF 144 COPYRIGHT 1993 ACS
AN CA111(1):3431n
TI Multi-analyte immunoassay
AU Ekins, Roger P.
CS Middlesex Sch. Med., Univ. Coll.
LO London W1N 8AA, UK
SO J. Pharm. Biomed. Anal., Volume Date 1988, 7(2), 155-68
SC 9-0 (Biochemical Methods)
DT J
CO JPBADA
IS 0731-7085
PY 1989
LA Eng
AB A review with 21 refs. Functionally specific and structurally specific assays; current developments in immunoassay methodol.; time-resolved fluorescence immunoassay; in vitro techniques of monoclonal antibody prodn.; ambient analyte immunoassay; and multi-analyte, radiometric immunoassays are described.

L2 ANSWER 29 OF 144 COPYRIGHT 1993 ACS
AN CA107(7):55001j
TI An overview of present and future ultrasensitive non-isotopic immunoassay development
AU Ekins, R. P.
CS Dep. Mol. Endocrinol., Middlesex Hosp. Med. Sch.
LO London, UK
SO Clin. Biochem. Rev. (Ultimo, Aust.), 8(1), 12-23

extent of binding of the [125I]T4 analog to the specific binding reagent was correlated with the free T4 concn. A sample contg. 20 pM free T4 and 3 nM oleic acid [112-80-1] would be interpreted as contg. 10.6 pM free T4, a bias of 47%. When the binding agent for the analog was added, a sample contg. 20 pg free T4/mL and 1 mM oleic acid would be interpreted as contg. 17 pg free T4/mL, a neg. bias of only 15%.

L2 ANSWER 45 OF 144 COPYRIGHT 1993 ACS
AN CA102(11):92247m
TI Non-isotopic immunoassay - an overview
AU Ekins, Roger; Jackson, Tom
CS Med. Sch., Middlesex Hosp.
LO London W1N 8AA, UK
SO Monoclonal Antibodies New Trends Immunoassays, Proc. Int. Symp. Radioimmunol., 6th, 149-63. Edited by: Bizollon, Charles A. Elsevier: Amsterdam, Neth.
SC 9-0 (Biochemical Methods)
DT C
CO 53DAAX
PY 1984
LA Eng
AB A review with 15 refs.

L2 ANSWER 56 OF 144 COPYRIGHT 1993 ACS
AN CA100(23):188424b
TI Measurement of analyte concentration
AU Ekins, Roger Philip
LO UK
SO PCT Int. Appl., 16 pp.
PI WO 8401031 A1 15 Mar 1984
DS W: AU, DK, FI, HU, JP, NO, SU, US
RW: AT, BE, CH, DE, FR, GB, LU, NL, SE
AI WO 83-GB210 26 Aug 1983
PRAI GB 82-24600 27 Aug 1982
IC G01N033-54; G01N033-74
SC 9-2 (Biochemical Methods)
SX 1, 2, 10
DT P
CO PIXXD2
PY 1984
LA Eng
AB Methods are described for detn. of biol. active substances such as drugs, viruses, and esp. hormones in body fluids without the need of measuring accurately the vol. of the sample. This method is potentially useful for designing a concn.-measuring device for

insertion into a body fluid of a living creature for in situ measurement. For example, antibody against hydrocortisone (I) was coupled to a solid support and was used to detn. I in std. solns. by RIA with 125I-labeled I. Results were satisfactory.

L2 ANSWER 57 OF 144 COPYRIGHT 1993 ACS
AN CA100(19):151147n
TI Interpretation of labeled-analog free hormone assay
AU Ekins, Roger; Edwards, Philip; Jackson, Tom; Geiseler, Dietrich
CS Med. Sch., Middlesex Hosp.
LO London W1N 8AA, UK
SO Clin. Chem. (Winston-Salem, N. C.), 30(3), 491-3
SC 2-1 (Mammalian Hormones)
DT J
CO CLCHAU
IS 0009-9147
PY 1984
LA Eng
AB Detns. of free thyroxine [51-48-9] by the Amerlex kit method are influenced by individual variations in serum levels of proteins that bind the labeled analog used in this test, as well as by variable concns. of substances that alter this binding. Thus, the binding of analog by antibody is not unaffected by serum constituents, and this system cannot be considered a generally valid indication of free hormone levels.

L2 ANSWER 58 OF 144 COPYRIGHT 1993 ACS
AN CA100(3):20062h
TI Method and composition for free ligand assays
AU Ekins, Roger Philip
LO UK
SO PCT Int. Appl., 28 pp.
PI WO 8303306 A1 29 Sep 1983
DS W: JP, US
RW: AT, BE, CH, DE, FR, GB, LU, NL, SE
AI WO 83-GB78 17 Mar 1983
PRAI GB 82-8043 19 Mar 1982
IC G01N033-54; G01N033-78
SC 9-2 (Biochemical Methods)
SX 2
DT P
CO PIXXD2
PY 1983
LA Eng
AB Immunol. methods are described for the direct detn. of free ligands (e.g. hormones, drugs, etc.) in samples (where the ligand is also

found sequestered to endogenous receptors) which simplify reagent prepn. and where the test receptor is directly or indirectly labeled. The method consists of combining the sample, labeled ligand receptor (antibody or protein A from Staphylococcus aureus labeled with 125I), and unlabeled differential binding ligand analog, incubating to permit the free ligand and unlabeled ligand analog to compete for the ligand receptor, sepg. the insol. ligand analog, detg. the amt. of ligand receptor bound to the ligand or ligand analog, and correlating the amt. of bound ligand receptor to the amt. of free ligand present in the sample. Thus, the method was used for the detn. of T4 in human serum following prepn. of 125I-labeled anti-T4 antibodies purified by affinity chromatog. and of Sepharose-bound T4-disuccinimidyl-bovine serum albumin (BSA) conjugate or of T4-disuccinimidyl-BSA conjugate coated on polypropylene test tubes.

L2 ANSWER 59 OF 144 COPYRIGHT 1993 ACS
AN CA99(23):190753n
TI Sensitive fluorescence immunoassay
AU Jackson, T.; Dakubu, S.; Ekins, R. P.
CS Med. Sch., Middlesex Hosp.
LO London W1, UK
SO Med. Lab. World, (Sept.), 23, 25-6, 28, 75
SC 9-0 (Biochemical Methods)
DT J
CO MLWODQ
IS 0140-3028
PY 1983
LA Eng
AB A review with 27 refs. esp. on the recent development of the time-resolved fluorescence immunoassay which is comparable to the radioimmunoassay in sensitivity.

L2 ANSWER 66 OF 144 COPYRIGHT 1993 ACS
AN CA96(13):100247m
TI Towards immunoassays of greater sensitivity, specificity and speed: an overview
AU Ekins, Roger
CS Inst. Nucl. Med., Middlesex Hosp. Med. Sch.
LO London W1N 8AA, UK
SO Symp. Giovanni Lorenzini Found., 11(Monoclonal Antibodies Dev. Immunoassay), 3-21
SC 9-0 (Biochemical Methods)
DT J
CO SGLFD9
IS 0166-1167